01/06/16

COMPETENT CELLS

Day two, grow in INNIS 18°C incubator

02/06/16

MINI PREP (PLASMID EXTRACTION)

The OD at 10am was 0.170. Wait until it raise to 0.5.
Then use sigma aldrich kit to do miniprep

03/06/16

COMPETENT CELLS

OD is 1.855. Dilute three times.
Day 3 of competent cells. All manipulations are made in cold room.

06/06/16

COMPETENCY TEST

Only 3 amount of DNA are tested: 5pg/µL, 20pg/µL and 50pg/µL. In each tube, we add 20µL of competent bacteria instead of 50µL, heated-shock 45s instead of 1min, use LB media instead of SOC, grow in 37°C incubator during 1h instead of 2h. Then grow overnight in 37°C incubator.

PCR PREPARATION

Our samples from the miniprep goes to the nanodrop (spectrometer): only 2 samples are good on 5. If bacteria get the plasmid, colonies turn pink.

1 (pink)	2 (pink)	3 (blank)	4 (blank)	5 (blank)
185,9 ng/L*10^-6 260/280= 1,84 260/230= 2,52	177,8 ng/L*10^-6 260/280= 1,84 260/230=2,51	18,4 ng/L*10^-6 260/280= 1,70 260/230= 50,51	14,3 ng/L*10^-6 260/280= 1,81 260/230=-7,68	15,1 ng/L*10^-6 260/280= 1,82 260/230= 7,20

07/06/16

COMPETENCY TEST

Results : Fail.

Transformation had failed or bacteria are not competent.

After discussion with Sabrina: bacteria was in stationary state (OD> expected) and so wasn't competent.

=> make competent cells again.

COMPETENT CELLS

Preparation of 1L SOB and TB. SOB goes to autoclave.

PCR

started at 11h45.

Electrophoresis started at 16h.

plasmid: pSB1C3 BBa-J04450

primers: VF2 et VR

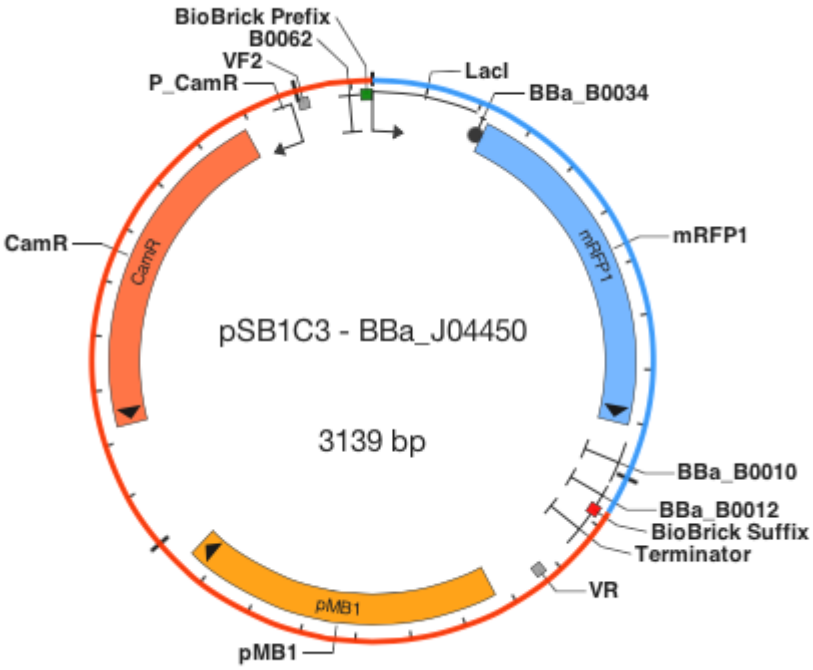
VF2: from 3000 to 3019

5' TGCCACCTGACGTAAGAA 3' ™= 54°C

VR: from 1243 to 1224

5' ATTACCGCCTTTGAGTGAGC 3' ™=60°C

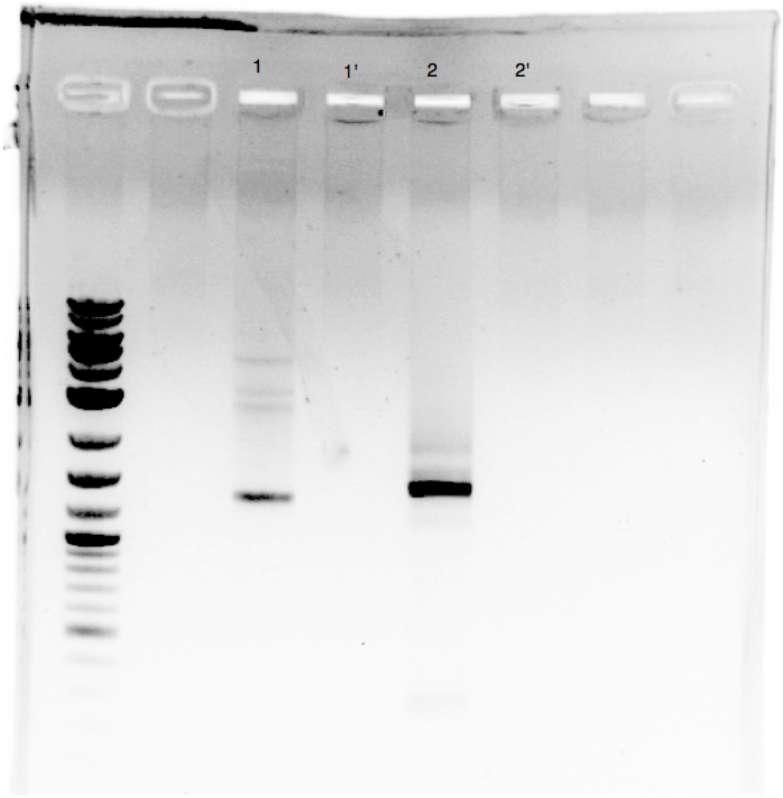
Amplified fragment size: 1243 pb



We don't care about RFP fragment because we just check if our miniprep are good and to train.

5 PCR tubes are prepared :

- two from culture 1 with 18.5ng/µL (1) in the first and 1.85ng/µL(1')
- two from culture 2 with 17.7ng/µL (2) and 1.77ng/µL (2')



Agarose gelelectrohoresis

We used log2 marker. The DNA from 1ng/µL wasn't enough to be amplify. Protocol from iGEM 2015 need to be modify to 20ng/µL.
Fragments size are as expected despite the fact that it had been contaminated.

08/06/2016

COMPETENT CELLS

day 1 → preculture + SOB

09/06/2016

COMPETENT CELLS

day 2 → preculture + TB : use phmeter from Teichman's team.

10/06/2016

COMPETENT CELLS

day 3 → OD:0.064, it is too low.
Incubation temperature rise to 29°C. Good OD obtain in the afternoon. Competent cells are stored at -80°C using liquid azote.

13/06/2016

COMPETENCY TEST

Transformed bacteria

14/06/2016:

COMPETENCY TEST

results :

- plate 50µg = 81 colonies
- plate 20µg = 101 colonies
- plate 5 µg = 9 colonies

→ maybe change over between plate 50µg and 20µg
→ number of colonies not enough
→ colonies aren't much colored

→ new transformation in order to check

PCR

Punc119: No results
→ wrong primers
→ new primers are order.

15/06/2016

COMPETENCY TEST

- Bacteria transformed the 13/06/2016 are back to incubation to 37°C.

Result: growth amazingly! We can consider them as competent cells at 48h incubation is enough.

  - Plate 50µg = 81 → 425 colonies
  - Plate 20µg = 101 → 605 colonies
  - Plate 5µg = 9 → 55 colonies

- Bacteria transformed the *14/06/2016*. Result:
  - Plate 50µg = 183 colonies
  - Plate 20µg = 34 colonies
  - Plate 5µg = 0 colonies

  Let them into incubation to see if it will happen same as bacteria from 13/06/2016

16/06/2016

COMPETENCY TEST

- Bacteria from 14/06/2016 grow as 13/06/2016

  Results:
  - Plate 50µg = 183 → 369 colonies
  - Plate 20µg = 34 → 101 colonies
  - Plate 5µg = 0 → 0 colonies

  not very convincing

Maybe should we try again with:

- a recovery avec period much longer and agitation.
- another plasmid with another antibiotic resistance to check the competency of our cells.

17/06/2016

MINIPREP

Mini for pSB1C3 plasmid.

Take some concentration measure at the nanodrop: 88,5 ng/µL.

260/280=1,88

260/230=2,73

The concentration seem to be very low. The kit we used was from last iGEM session (2015). But the RNases wasn't storage at the fridge and washing solution may have ethanol evaporate. If there is no enough ethanol, washing solution may have elute much of the DNA.

=> The low amount of plasmid may be due to the old kit used for the mini-prep. We will compare this kit to the lab kit.

PCR

Miniprep → Backbones

PCR protocol:

x 1:

5x Phusion GC buffer: 10 uL

10 mM dNTP: 1 uL

10 uM forward primer: 2,5 uL

10 uM reverse primer: 2,5 uL

Phusion DNA pol: 0,5 uL

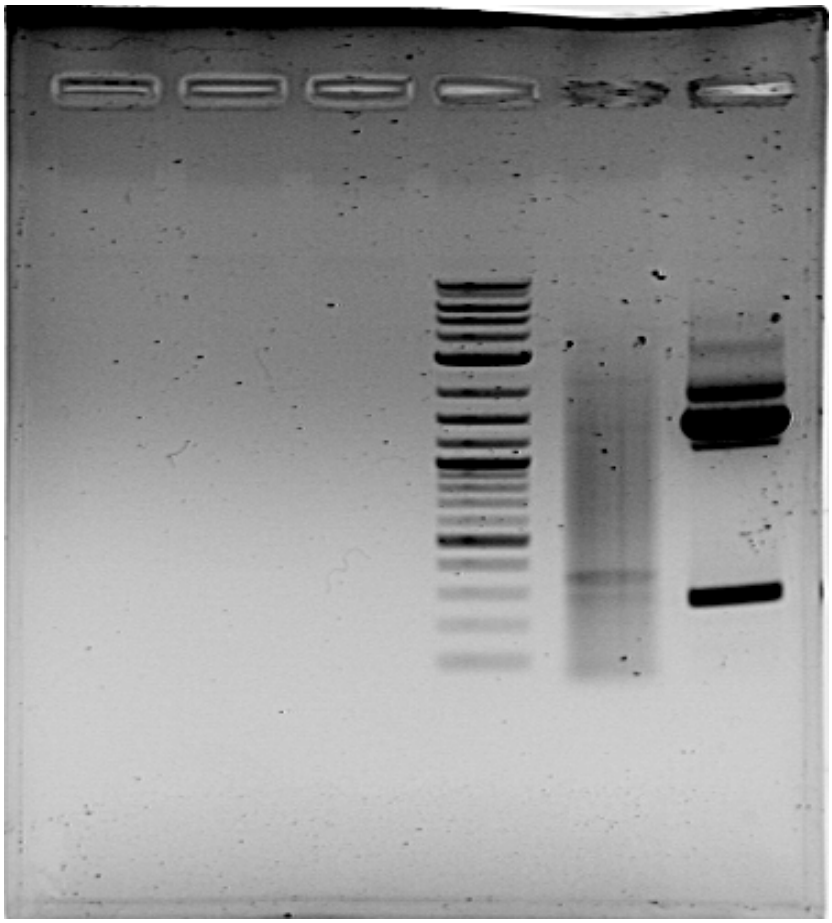
Plasmid: 1 uL (20 ng)

H2O: add until 50 uL

Control: replace DNA by H2O

PCR program:

Initial denaturation: 94°C 5min
denaturation: 94°C 30s
annealing: 55°C 30s
elongation: 72°C 55s
final extension: 72°C 10min
final hdd: 15°C temps infini



Agarose gelelectrophoresis

There was a contamination of our PCR products. On Monday we need to do some PCR to know where comes from the contamination.

20/06/2016

PCR

We must make sure that the contamination doesn't comes from the mix. We need to test: the buffer, primers, dNTPs and water. In order to test is, we did a PCR for each tubes which there is a new constituent and a fifth tube for the control. We mustn't put DNA in tubes but water, then add the enzyme.

For 1 mix:
5x Phusion GC buffer: 10 µL
10 mM dNTP: 1 uL
10 µM forward primer: 2,5 µL
10 µM reverse primer: 2,5 µL
Phusion DNA pol: 0,5 µL
Plasmid: 1 µL (20 ng) but here replace water by 1 µL of H2O
H2O: fill until 50 µL

	tube 1	tube 2	tube 3	tube 4	tube 5
Buffer	new	old	old	old	new
Primer RF	old	new	old	old	new
Primer RV	old	new	old	old	new
dNTP	old	new	new	old	new
H2O	old	old	old	new	new

21/06/2016

PCR

Electrophoresis from Monday 20 June in order to know where was the contamination.
result:
The contamination comes from dNTPs because in the third tube which contain new dNTPs, there is no signal. It is the same case for the control (tube 5).

22/06/2016

Linejy has arrived

MINI-PREP

Made a mini-prep using the lab kit to compare results with our kit. We hope to collect more plasmid.

nanodrop results:

concentration:136,8 ng/ul

260/280= 1,84

260/230=2,21

results obtained with iGEM kit:

concentration : 140,2 ng/uL

260/280 = 1,85

260/230 = 2,48

After these results, no need to order another kit.

PCR NLP22

DNA concentration : 37,1 ng/µL

Need to be diluted to obtain 10ng/µL and to pick out 1µL for the PCR .

Dilution: 8 µl H2O + 2 µl plasmid.

PCR made 3 times (replicates) plus one negative control.

PCR protocol

mix: x 4

H2O: 130 uL

5x Phusion GC buffer: 40 uL

10 mM dNTP:4 uL

10 uM forward primer: 10 uL

10 uM reverse primer: 10 uL

Phusion DNA pol: 2 uL

Then add 1 µL (10ng) plasmid with NLP22 in each tube (3 tubes) and add 1 µL H2O in control.

Program

Initial denaturation: 94°C 5min

denaturation: 94°C 30s

annealing: 63°C 30s

elongation: 72°C 15s

final extention: 72°C 10min

final hdd: 15°C temps infini

Agarose gelelectrophoresis

23/06/2016

PCR DSIP

Concentration DNA: 22,4 ng/uL
Dilute it to have 10ng/µL and take 1µL for the PCR .

Primer dilute : 10µL primers + 90µL water ⇒ Vf = 100µL

Mix

- Buffer
- DNTP
- DNAp
- primer Fow et Rev
- water
- DNA

1 Marker
2 negative controls
3,4,5 DSiP

PROGRAM

Initial denaturation: 94°C 5min
denaturation: 94°C 30s
annealing: 67°C 30s
elongation: 72°C 6s
final extention: 72°C 10min
final hdd: 15°C temps infini

PCR pFRq

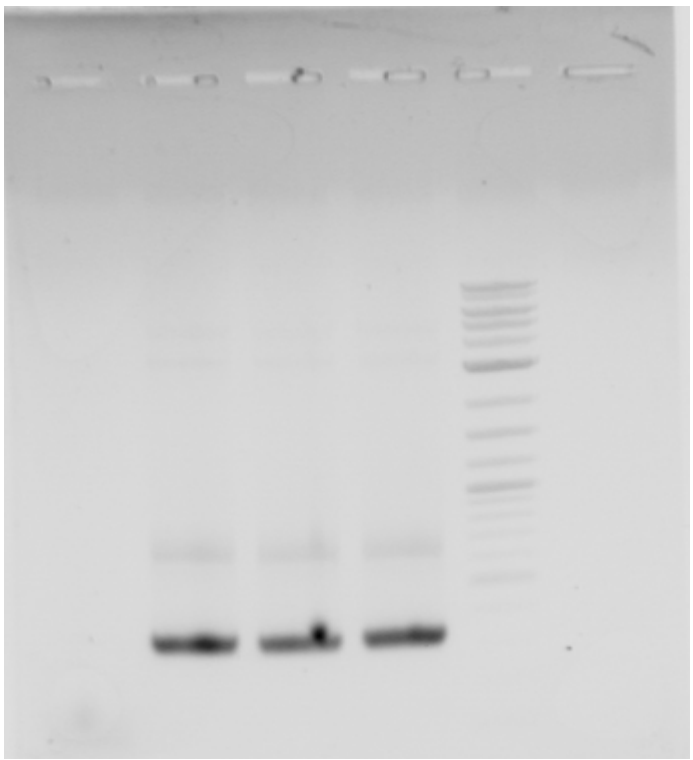
concentration: 31,2 ng/µL
Dilute it to have 10ng/µL and take 1µL for the PCR .

H2O in control.

H2O: 130 µL
5x Phusion GC buffer: 40 µL
10 mM dNTP:4 µL
10 µM forward primer: 10 µL
10 µM reverse primer: 10 µL
Phusion DNA pol: 2 µL
Then add 1 µL (10ng) plasmid with pRFq in each tube (3 tubes) and add 1 µL

PROGRAM

Initial denaturation: 94°C 5min
denaturation: 94°C 30s
annealing: 68°C 30s
elongation: 72°C 9s
final extention: 72°C 10min
final hdd: 15°C infinite time



Agarose gelelectrophoresis

24/06/16

DSIP PCR

DSiP:
Initial concentration : 22,4 ng/µL
Final concentration : 5 ng/µL

Dilute DSIP tube = 2µL initial solution picked out + 8 µL H2O

Mix PCR (x2)

- H20 = 65µL
- x5 Buffer = 20 µL
- dNTP = 2µL
- Fow et Rev = 5 µL
- ADNp (phusion) = 1µL
- ADN = 1µL

Initial denaturation: 94°C 5min
denaturation: 94°C 30s
annealing: 55°C 30s
elongation: 72°C 6s
final extension: 72°C 10min
final hdd: 15°C infinite time

NB : Temperature for hybridization were too hot, we should use primers PCR but lower because of floating ending.
Plasmid : 55°C
gDNA : 60°C

30/06/16

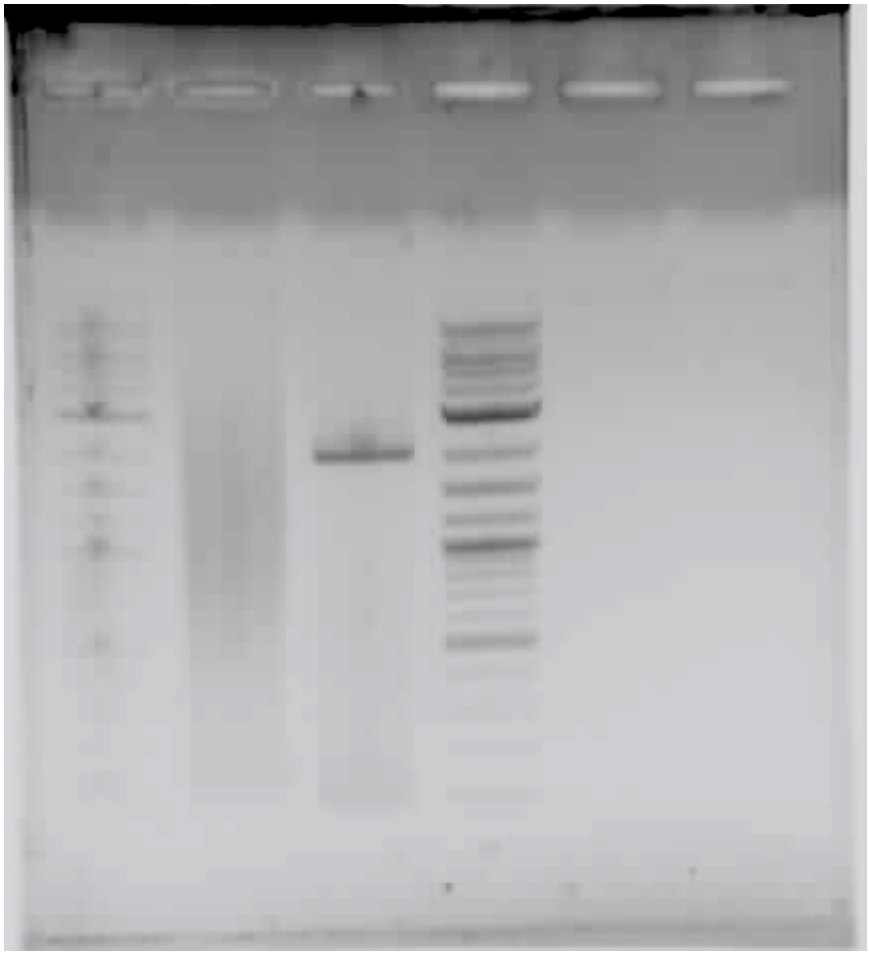
PCR pUNC119

Mix PCR (x2)

H20 = 65µL
x5 Buffer = 20 µL
dNTP = 2µL
Fow et Rev = 5 µL
ADNp (phusion) = 1µL
ADNg = 1µL et 1µL of H2O in control tube

Program PCR

Initial denaturation: 94°C 5min
denaturation: 94°C 30s
annealing: 60°C 30s
elongation: 72°C 1min 10s
final extension: 72°C 10min
final hdd: 15°C infinite time



Agarose gelelectrophoresis

plasmid transformation IDT

- Pipette 50µl of competent cells into 2ml tube
- Add 1µL DNA into 2ml tube
- Close 2ml tubes, incubate on ice for 30min
- Heat shock tubes at 42°C for 1 min.
- Incubate on ice for 5min
- Add 200µL LB
- incubate 1h at 37°C

At the same time, prepare “petri boxes” for 3 boxes

16 mL / box

50 ml LB (solid) + 25µL d’ampicilin (x2000) (do it in sterilize conditions)

- after incubation put in each boxes 230µL on the middle of the box and spread it (in sterilize conditions)